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The effect of chemical irritation on the respiration of fungi

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The effect of chemical irritation upon the growth of fungi has been the subject of considerable study for a number of years past. The results obtained by Raulin, [*] Richards, [†] Ono, [‡] Hattori, [§] and others have gone to prove that certain metallic salts, in small quantities, stimulate the metabolic activity of various fungi and thereby induce a more rapid growth than is normal. This action is interpreted by Richards [||] as being a means by which the plant is enabled to dispose more economically of the sugar or other source of the carbohydrates required in growth.

The fungi used by Richards in his experiments were *Sterigmatocystis* (*Aspergillus*) *niger*, *Penicillium glaucum*, and *Botrytis*, and the substances which served as irritants were zinc, iron, cobalt and nickel sulphates, NaFl, LiCl, Na_2SiO_3, cocaine, morphine and amygdalin. Ono made use of the same irritants with the addition of $HgCl_2$. Hattori experimented with $CuSO_4$; Yasuda [¶] with certain alkaloids; Hueppe [**] found the activities of bacteria stimulated by certain poisons. Schultz [††] likewise observed an acceleration of fermentation in yeast following the addition of small quantities of poisonous substances, but whether this was due to a more rapid growth and division, or to greater activity of the individual cells, has not been determined.

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[*] Raulin, J. Études chimiques sur la végétation; deuxième partie. Ann. Sci. Nat. Bot. V. 11: 190–299. 1869.

[†] Richards, H. M. Die Beeinflussung des Wachsthums einiger Pilze durch chemische Reize. Jahrb. Wiss. Bot. 30: 665–688. 1897.

[‡] Ono, N. Ueber die Wachsthumsbeschleunigung einiger Algen und Pilze durch chemische Reize. Jour. Coll. Sci. Imp. Univ. Tokyo, 13: 141–186. 1900.

[§] Hattori, H. Studien über die Einwirkung des Kupfersulfats auf einige Pflanzen. Jour. Coll. Sci. Imp. Univ. Tokyo, 15: 371–394. 1901.

[||] Richards, H. M. The effect of chemical irritation on the economic coefficient of sugar. Bull. Torrey Club, 26: 463–479. 1899.

[¶] Yasuda, A. On the effect of alkaloids upon some moulds. Bot. Mag. Tokyo, 15: 79–83. 1901.

[**] Hueppe, F. Naturwissenschaftliche Einführung in die Bakteriologie, 55. Wiesbaden, 1896. (Not seen.)

[††] Schultz, H. Pflüger's Archiv, 42: —. 1888. (Reference in Bot. Zeitung, 46: 610. 1888.)

Experiments were likewise performed by Ono on algae with like results except that the optimum concentration of the stimulants was much smaller. Similar work has been done on the phanerogams by Copeland and Kahlenberg * using pure metals as stimulants, by Townsend † with ether and HCl, and by Kahlenberg and True. ‡ Chapin § also found an increase in the growth of certain seedlings due to the presence in the atmosphere of small amounts of CO_2, larger quantities of which acted as a poison.

In all of these cases the amount of stimulation is indicated by the increase in the plant body, as shown by dry weight in the case of the fungi and algae, and by the increased surface of the organs of phanerogams. It is well known, however, ‖ that only a part of the nutrient material absorbed by the plant is used to build up its living substance ; a part is continually being oxidized to provide the necessary energy for carrying on life-processes. Fully to determine, therefore, the relation between the plant and its food-supply, one would have to take into account both the amount of the latter which is used as constructive material, measured by the increased weight or size of the plant, and that which undergoes katabolic changes during which CO_2 and other waste products are liberated.

Richards' experiments were concerned with the first factor, that is, the amount of dry substance produced and its relation to the amount of sugar used, or the economic coefficient. Ono confirmed his results, and in addition he determined the relative quantities of oxalic acid produced by fungi under normal conditions and under stimulation, finding a decrease in the latter case with all substances tried except $NiSO_4$. To account for this Ono suggests

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* Copeland, E. B., and Kahlenberg, L. The influence of the presence of pure metals upon plants. Trans. Wisc. Acad. **12** : 454–474. 1899.

† Townsend, C. O. The correlation of growth under the influence of injuries. Ann. Bot. **11** : 509–532. 1897. — The effect of hydrocyanic acid gas upon grains and other seeds. Bot. Gaz. **31** : 241–264. 1901.

‡ Kahlenberg, L., and True, R. H. On the toxic action of dissolved salts and their electrolytic dissociation. Bot. Gaz. **22** : 81–124. 1896.

§ Chapin, P. Einfluss der Kohlensäure auf das Wachsthum. Flora, **91** : 348–379. 1902.

‖ Pfeffer, W. Physiology of plants, ed. 2, **1** : 287, 523. 1900.

that the carbohydrate which during normal growth is decomposed, in the processes of metabolism, to form oxalic acid, is not so transformed during the accelerated growth resulting from stimulation, but is immediately changed into constructive material for the plant body. Of this, the decrease in the economic coefficient (*i. e.*, ratio of dry weight to sugar used) of the stimulated cultures seems an indication. Oxalic acid is not, however, the only waste product of metabolism. Since, according to Pfeffer,[*] "a decreased formation of oxalic or other organic acids is accompanied by an increase in the ratio of CO_2 exhaled to the oxygen absorbed," the amount of CO_2 given off under the varying conditions must also be reckoned with; it must be determined, that is, whether all the additional carbohydrate normally used to produce oxalic acid is now transformed into formative substance, or whether part of it may be wholly oxidized to produce larger quantities of CO_2. This ratio of the dry substance produced to the amount of CO_2 exhaled, or the respiratory quotient, was found by Kunstmann [†] to vary with changing conditions, decreasing with a rise of temperature or with higher concentrations of the nutrient solution. The effect of chemical stimulation was not included among the conditions.

The effect of such stimulation upon respiration alone without regard to the possible acceleration of growth ensuing was studied by Jacobi,[†] who obtained an increase in amount of CO_2 produced by certain phanerogams, due to the influence of KCl, NaCl, KNO_3, iodine, chinin, antipyrin, oxalic acid and various organic substances. Copeland [‡] obtained similar results; and likewise Kosinski [§] in the case of fungi. Morkowin [||] found a decided increase in the intensity of respiration, both normal and intramolecular, of etiolated leaves and shoots under the influence of ether, alcohol and various alkaloids. Pfeffer states [*] that according to Elfving and Lauren adult parts of higher plants, where stimulation is unaccompanied by growth, react in the same manner to a short treatment with ether and chloroform ; he notes however that Detmer and also Bonnier and Mangin found no such reaction.

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[*] Pfeffer, W. Physiology of plants, ed. 2, 1 : 527. 1900.

[†] Kunstmann, H. Ueber das Verhältnis zwischen Pilzernte und verbrauchter Nahrung. Leipzig. 1895. [Inaug. Diss.]

[‡] Jacobi, B. Ueber den Einfluss verschiedener Substanzen auf die Athmung und Assimilation submerser Pflanzen. Flora, 86 : 289–327. 1899.

[§] Copeland, Bot. Gaz. 35 : 81–98, 160–183. 1903.

[||] Kosinski, I. Die Athmung bei Hungerzuständen und unter Einwirkung von mechanischen und chemischen Reizmittteln bei *Aspergillus niger*. Jahrb. Wiss. Bot. 37 : 137–204. 1901.

[¶] Morkowin, N. Recherches sur l'influence des anesthésiques sur la respiration des plantes. Revue Gén. de Bot. 11 : 289–303, 341–352. 1899. — Ueber den Einfluss der Reizwirkunzen auf die intramoleculare Athmung der Pflanzen. Ber. Deut. Bot. Ges. 21 : 72. 1903.

Summarizing then, the results quoted, we find that small quantities of certain poisonous substances act as stimulants, increasing the growth of certain plants ; they also increase respiration, but what relation the latter increase bears to the former is not determined. Since, however, in the case of fungi, it has been found that stimulation raises the economic coefficient of the sugar, *i. e.*, allows the plant to make use of the sugar to form a greater amount of dry substance in a given time, the question of the amount of CO_2 which is produced at the same time becomes one of interest.

This problem was suggested to me by Dr. H. M. Richards, to whom I wish to express my gratitude for his interest and valuable assistance. The work was done in the botanical laboratory of Barnard College during the winters of 1901–1902 and 1902–1903.

Two kinds of apparatus have been employed in carrying on this work, one that of Godlewski described by Kunstmann [†] the other a modification of the Pfeffer-Pettenkofer apparatus. In all the experiments, however, the fungi used were *Sterigmatocystis nigra* and *Penicillium glaucum*. The formula for the nutrient solution was that given by Richards : [‡]

KH_2PO_4	0.05g.
$MgSO_4$	0.25g.
NH_4NO_3	1.00g.
$FeSO_4$	trace.
Sugar	5.00g.
Water	100 cm.

Only the purest chemicals and sugar obtainable were used, and the water was twice distilled. The dishes and flasks in which the cultures were grown were rinsed with dilute HCl, carefully washed with double-distilled water and dried in a dry sterilizer. The spores were sown by rubbing small pieces of glass rod, exactly equal in size, upon the dry stock supply, and then transferring them to the dishes by means of glass tipped forceps. It was found convenient to keep a supply of the concentrated nutrient solution on hand, containing all the ingredients except sugar. The necessary amount was diluted and the sugar added when the series was set up.

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[*] Pfeffer, W. Physiology of plants, Ed. 2, **1** : 564. 1900.

[†] Kunstmann, *l. c.* 10. 1895.

[‡] Richards. Jahrb. Wiss. Bot. **30** : 667. 1897.

At first the Godlewski apparatus used by Kunstmann was employed. This consisted, in brief, of a bell-jar enclosing two crystallizing dishes; the smaller of these held the fungus growing on 75 c.c. of the nutrient solution, and rested upon a glass strip placed across the larger dish which contained 150 c.c. of a 10 per cent. solution of KOH. The mouth of the jar was closed with a rubber cork provided with two holes through one of which passed a thermometer, and through the other a glass tube leading to a mercury valve, with which, in turn, was connected a KOH tube in such a way that air could pass into the jar, but could not escape from it. The bell-jar was sealed to a ground glass plate by means of vaseline and the joining was then painted with two coats of shellac to make it water tight. Three jars were used, one to serve as a control, and two for the experiment; the results given are, unless otherwise stated, the average of the two grown at the same time and under the same conditions. The greatest difficulty was experienced in keeping the cultures at an even temperature, approximately near the optimum for *Sterigmatocystis*, which is 30–34° C. The plan finally followed was to place all three jars in a zinc trough partly filled with water, the temperature of which would change more slowly than that of the air, and then to enclose the whole in a wooden box placed near a steam radiator. Under these conditions the temperature was raised to the neighborhood of 30° C., and varied not more than three or four degrees during any one experiment.

At the close of the experiment the fungus was killed with formalin as soon as the jar was opened, washed with distilled water, collected in weighed filter papers, dried in an oven at a temperature of 70° C., and after cooling in a dessicator was carefully weighed. The KOH was diluted to 250 c.c.; the CO_2 was precipitated out of it by an equal quantity of barium hydrate, and the alkalinity of the remaining KOH was determined by titrating in the usual way with HCl of such strength that 1 c.c. of the acid = 1 mg. of CO_2. The result was then subtracted from that obtained by treating a certain amount of the stock KOH in the same way, and thus the amount of CO_2 given off by the fungus was obtained. The results are given in milligrams both for the weight of the fungus and the amount of CO_2 given off.

At the same time a series of experiments was carried on with a form of the Pfeffer-Pettenkofer respiration apparatus. The particular object in using this was to determine the rate of respiration during short intervals of time. Certain practical difficulties made this impossible, however, at that time, and the attempt was abandoned. Later, the same apparatus was started again in a slightly different way; *i. e.*, a strong solution of KOH was used and the same experiment was continued for a week at a time. It was set up in the dark room, which, being small and kept carefully closed, could be maintained at a comparatively even temperature in the neighborhood of 24° C.

The air in entering the apparatus passed through three U-tubes filled with pumice stone wet with a strong solution of KOH which was frequently renewed. A $Ba(OH)_2$ test-bottle was placed in the course to prove that no CO_2 passed over to the cultures, and the air was then distributed to these, four in number, by means of a T-tube and two Y-tubes. The cultures were grown in Erbenmeyer flasks of about 250 c.c. capacity, in which only 75 c.c. of the nutrient solution was used. This brought the film of mycelium at the level of the widest diameter of the flask and the flasks were all as nearly as possible of the same diameter. Between the culture flasks and the Pettenkofer tubes were interposed large test-tubes to prevent any possible backward flow of the KOH into the culture flasks; 150 c.c. of a 10 per cent. solution of KOH was introduced into the absorption tubes by means of a large pipette; the stock solution was kept in a tightly stoppered bottle sealed with vaseline. At the exit end of the tubes were placed screw pinch-cocks to regulate the amount of air drawn through the apparatus, which was measured by the size and rate of flow of the bubbles of air in the tubes. Any slight difference in the rate of flow would be of no importance when the length of time during which the experiment ran — six to nine days — is taken into consideration. Between the tubes and the aspirator were inserted $Ba(OH)_2$ test-bottles. The aspirator was a simple one fastened to the water faucet.

At the close of the experiment the aspirator was shut off, the stop-cocks closed, the cultures immediately killed with formalin, and the contents of the tubes quickly emptied into bottles which were tightly corked. The amount of CO_2 absorbed was obtained by titration as before.

The metallic salts used as irritants in these experiments were $ZnSO_4$, $FeSO_4$ and LiCl. In each case such a concentration was used as had been found by Richards to be most favorable for an increased growth of the fungus. A 1 per cent. stock solution of anhydrous $ZnSO_4$ was made carefully; of this, .3 c.c. was used for 75 c.c. of the nutrient solution, giving thus a .004 per cent. solution or, expressed in terms of a normal (gram equivalent) solution,* .00049; of the $FeSO_4$, a .082 per cent. solution was used, or .01078 normal; and of LiCl, a .162 per cent. solution or .03822 normal.

In the following tables the results of the experiments are presented. In the first are given five series carried on with the Kunstmann apparatus, for three or four days at a temperature of 28°–31° C. The irritant was $ZnSO_4$. In the first column are given the dry weights of the cultures, both normal and irritated; in the second the amount of CO_2 given off by each, expressed in millegrams; and in the third the ratio of the weight to the CO_2. On examination of these figures it is evident that the addition of the zinc has caused an increase in the growth of the fungus and there has been likewise an increase in the amount of CO_2 respired. The amount of CO_2 respired is sometimes less than the corresponding weight of the culture, sometimes more, but when one compares the ratios of the two cultures in any one series, one finds very little difference between them; that is, the increase in respiration has been correspondingly great in both, with perhaps a slight advantage on the side of the irritated fungus. There is one exception to this, the fourth series, where the difference is unaccountably great, owing to an unusually large evolution of CO_2 from the stimulated culture. The increase in the amount of CO_2 given off is therefore but little greater than might naturally be expected from the increased respiratory surface due to the greater growth of the fungus.

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* Livingston, B. E. The role of diffusion and osmotic pressure in plants, 22. 1903.

The second table shows the results of five experiments performed with the Pettenkofer apparatus; the cultures, being grown at a lower temperature, 20–24° C., were allowed to run for a longer time: five, six, or seven days. In these, the figures for the irritated fungus represent the average of three cultures, grown under precisely similar conditions. Here too the ratios are very near one another. It is noticeable however that the difference, such as it is, is reversed in the second table, in all but one case, and the respiration of the normal grown in the Pettenkofer apparatus is therefore apparently a little greater, relatively, than that of the irritated fungus. The cause of this reversal of the ratios obtained by the two methods I have not been able to determine, but the difference is too slight to be of any importance. In Tables IX and X are given the same figures as in I and II, but the averages are found for the weights, CO_2, and ratios of each set of series; that is, for the series obtained by the Kunstmann method (Table IX), the average ratio of weight to CO_2 for the normal is .98, for the stimulated culture is 1.18; for those in the Pettenkofer apparatus (Table X) the average is 1.16 for the control as against .94 for the stimulated fungus.

Bearing these figures in mind, let us refer again to Ono's experiments in determining the amount of oxalic acid produced by fungi during growth under stimulation. With all the irritants used, with the exception of $NiSO_4$, he found a more or less marked decrease in the production of oxalic acid, a decrease shown (to quote some of his figures) * by the difference between 2.58 acid for every gram of dry weight of the normal, and 0.428 for the culture to which has been added .003 per cent $ZnSO_4$. The duration of this experiment was twenty-seven days and the temperature 16–20° C. Ono suggests three possible explanations for this de-

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* Ono, *l. c.* 174. 1900.

crease:[*] (1) Although oxalic acid is a normal product of metabolism of the fungus, it is to be regarded nevertheless as a product of incomplete oxidation, and if the metabolic activity were increased, more CO_2 might be produced and therefore less oxalic acid. (2) The same result would occur in case the acid should disappear through a further "working-over" on the part of the fungus. Thereby it could be taken up again as building material or be decomposed without being useful to the fungus. Yet, as shown by Wehmer,[†] oxalic acid is poor food stuff for *Aspergillus*, and in the presence of a plentiful supply of sugar would probably remain intact. (3) Those materials (carbohydrates, etc.) which in normal growth are, by metabolism, transformed in part into oxalic acid, in consequence of increase in growth due to the stimulation are not separated out in that form, but are changed directly into the fungus body, in short, are applied as formative material.

Of these three possibilities, Ono concludes from his experiments that the third is the true explanation, since in every case the economic coefficient was much greater in the control than in the stimulated fungus. "Dieses Verhältnis deutet also an, das die Pilze bei Anvesenheit von Zinksulfat veranlasst wurden, mit einem verhältnismässig kleinen Verbrauch von Zucker eine bedeutend grössere körpersubstanz aufbauen zu können." [‡] This was also the conclusion reached previously by Richards. [§]

Further support is now given to this explanation by the results presented here, since Ono's first suggestion is thereby eliminated; that is, the present figures show that an amount of CO_2 is produced by the stimulated fungus which varies a little, being either more or less relatively to the weight of the fungus than that produced by the normal, but still remains comparatively close to the latter. The increase in metabolic activity has, therefore, not increased (relatively) the production of CO_2 although the formation of oxalic acid under similar conditions was materially checked, and the carbohydrates formerly used in the manufacture of the latter must consequently be devoted to the building up of new substance in the plant.

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[*] *Idem*, 157. 1900.

[†] Wehmer, Bot. Zeitung, 49: 553. 1891.

[‡] Ono, *l. c.* 160. 1900.

[§] Richards, H. M. The effect of chemical irritation on the economic coefficient of sugar. Bull. Torrey Club. **26**: 463–479. 1899.

In Table III two series are shown, in which glycerine was used instead of sugar as the source of the carbohydrate. All of the figures here are rather low, owing to the slower growth of *Sterigmatocystis* in such a nutrient medium; the ratios, however, bear much the same relation to one another as in the preceding series, .61 and .69 (normal) to .77 and .68 (stimulated), respectively.

In the experiments recorded in Table IV, .082 per cent. (.01078 normal) $FeSO_4$ was used as the stimulant, since this concentration is near that which caused the greatest increase in growth in Richards' experiments. A .162 per cent. (.0381 normal) solution of LiCl was also tried (Tables V and VI), and although the increase in growth was not as great as might have been expected from Richards' results, yet the evolution of CO_2 bears much the same relation to it as in the preceding cases.

A few experiments were likewise made with *Penicillium glaucum.* Though *Penicillium* grows well at a lower temperature than does *Sterigmatocystis*, yet it grows slowly, and the total weight of the normal is seldom as great as is that of *Sterigmatocystis* in the majority of cases. The evolution of CO_2 is nevertheless very active, and the number of milligrams of CO_2 given off exceeds that of the dry weight of the fungus. Several of these cultures were raised on the usual nutrient medium (Table VII), and one (Table VIII) on a solution in which .5 gr. asparagin replaced the NH_4NO_3 as the source of nitrogen. The weight of the irritated fungus in the latter case was much greater than that of the normal, but the evolution of CO_2 kept pace with it to such an extent that the ratio is 1.22 as compared with 1.26 in the normal.

In reviewing, then, the results recorded here, it is evident that the addition of small quantities of $ZnSO_4$, $FeSO_4$ and LiCl has increased the rate of growth of *Sterigmatocystis* and *Penicillium* so that a larger amount of dry substance is produced within a given time than in normal cultures; that at the same time there has been an increase in the production of CO_2, an increase not too great, however, to be accounted for by the enlarged area of the plant. The ratio of dry weight to CO_2 of the irritated fungus is therefore approximately equal to that of the normal. Taking into account previous results which show that the effect of the addition of such poisons is to enable the plant to make more economical use of the carbohydrates supplied to it, we can interpret this action as a stimulus which causes the fungus to transform more of the food material into its own substance, and less into waste products such as oxalic acid, while at the same time the respiration remains relatively unchanged.

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TABLE I.

Kunstmann apparatus.

Sterigmatocystis nigra. 5 per cent. sugar solution.

A = Normal. B = average of two cultures to which .004 per cent.* or .00049 normal (gram equivalent) $ZnSO_4$ has been added. Temperature 28–30°C.

		Weight of Fungus.	CO_2	Ratio.	Time.
I.	A	335.2	155	.46	3 days.
	B	597.6	392.5	.67	
II.	A	398.5	455	1.13	3 "
	B	607.1	755	1.27	
III.	A	479.7	705	1.46	4 "
	B	805.1	1185	1.47	
IV.	A	480.8	415	.86	4 "
	B	783.7	1230	1.56	
V.	A	533.6	465	.87	4 "
	B	875.6	780	.89	

TABLE II.

Pettenkofer apparatus.

Same as in Table I, except that B = average of *three* cultures to which .004 per cent.,* (.00049 normal) $ZnSO_4$ has been added. Temperature 20–24° C.

		Weight of Fungus.	CO_2	Ratio.	Time.
VI.	A	600	420	.70	7 days
	B	1138.1	987	.86	
VII.	A	673.6	768	1.14	7 "
	B	888.0	888	1.00	
VIII.	A	885.5	1173	1.32	7 "
	B	1086.6	1143	1.05	
IX.	A	1056.0	978	.92	6 "
	B	1237.2	1036	.83	
X.	A	715	936	1.30	5 "
	B	1327	1263	.95	

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* Calculated as anhydrous salt.

Table III.

Pettenkofer apparatus.

Nutrient solution a 5 per cent. glycerine instead of sugar; otherwise as in Table II. B = .004 per cent.* (.00049 normal) $ZnSO_4$.

		Weight.	CO_2	Ratio.	Time.	Temp.
XI.	A	489	300	.61	7 days.	23–25°
	B	1194.7	920	.77		
XII.	A	358.5	249	.69	7 "	20–24°
	B	724.3	498	.68		

Table IV.

Pettenkofer apparatus.

Same as in Table II, but B = .082 per cent.* (.01078 normal) $FeSO_4$.

		Weight.	CO_2	Ratio.	Time.	Temp.
XIII.	A	1142.5	867	.75	6 days.	26–29°
	B	1691	1513	.89		
XIV.	A	687	180	.26	6 "	
	B	1449.4	830	.57		

Table V.

Kunstmann apparatus.

Same as in Table I, but B = .162 per cent.* (.0381 normal) LiCl.

B here was not an average of two, but a single culture. Temperature 19–28° C.

		Weight of Fungus.	CO_2	Ratio.	Time.
XV.	A	615.5	514	.83	11 days
	B	775.5	814	1.04	

Table VI.

Pettenkofer apparatus.

Same as in Table II, but B = .162 per cent.* (.0381 normal) LiCl.

		Wght. Fungus.	CO_2	Ratio.	Time	Temp.
XVI.	A	1434	1110	.78	7 days	25°C.
	B	1501.1	1311	.87		
XVII.	A	679	645	.94	8 "	22°–27°C.
	B	723.7	747	1.03		

* Calculated as anhydrous salt.

Table VII.

Pettenkofer apparatus.

Penicillium glaucum. Otherwise as in Table II. B = .004 per cent.* (.00049 normal) $ZnSO_4$.

		Wght. Fungus.	CO_2	Ratio.	Time.	Temp.
XVIII.	A	489.50	705	1.43	7 days	18–22°C.
	B	525.75	739.5	1.40		
XIX.	A	438.5	729	1.66	9 "	19–22°C.
	B	539.33	991	1.66		

Table VIII.

Pettenkofer apparatus.

Penicillium glaucum.

Same as in Table VII., but .5 grams asparagin used in place of NH_4NO_3. B = .004 per cent.* (.00049 normal) $ZnSO_4$.

		Weight.	CO_2	Ratio.	Time.	Temp.
XX.	A	354.5	450	1.26	9 days.	17.5–20°
	B	932.16	1145	1.22		

Table IX.

Kunstmann apparatus. Average of five series shown in Table I. .004 per cent.* (.00049 normal) $ZnSO_4$.

	Normal.			Average of 2.		
	Weight.	CO_2	Ratio.	Weight.	CO_2	Ratio.
I.	335.2	155	.46	597.6	392.5	.67
II.	398.5	455	1.13	607.1	755	1.27
III.	479.7	705	1.46	805.1	1185	1.47
IV.	480.8	415	.86	783.7	1230	1.56
V.	533.6	465	.87	875.6	780	.89
Average.	445.56	439	.98	733.8	868.5	1.18

Table X.

Pettenkofer apparatus. Average of five series shown in Table II .004 per cent.* (.00049 normal) $ZnSO_4$.

	Normal.			Average of 3.		
	Weight.	CO_2	Ratio.	Weight.	CO_2	Ratio.
VI.	600	420	.70	1138.1	987	.86
VII.	673.6	768	1.14	888.0	888	1.00
VIII.	885.5	1173	1.32	1086.6	1143	1.05
IX.	1056.0	978	.92	1237.2	1036	.83
X.	715	936	1.30	1327.0	1263	.95
Average.	759.4	885.37	1.16	1207.23	1138.2	.94

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* Calculated as anhydrous salt.